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Differential expression of five sialyltransferase genes in human tissues.

Kitagawa H, Paulson JC.

Cytel Corporation, San Diego, California.

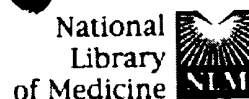
Cell type-specific expression of specific carbohydrate structures on cell surface glycoproteins and glycolipids is increasingly recognized for providing information relevant to cell-cell interactions in developing and adult organisms. Sialyltransferases contribute to the diversity in carbohydrate structure through their attachment of sialic acid in various terminal positions on glycolipid and on glycoprotein (N-linked and O-linked) carbohydrate groups. In this report, differential expression of five sialyltransferase genes in human tissues is evaluated as a potential mechanism to account for cell type-specific variation in terminal sialoside structures produced by a cell. For this analysis, the complete cDNA of the human Gal beta 1,3GalNAc alpha 2,3-sialyltransferase and a partial cDNA of the developmentally regulated STX gene were cloned. Northern analysis was performed using these cDNAs and those of three previously cloned human sialyltransferase genes as probes. Each of the five sialyltransferase genes exhibits dramatic differential expression in the 16 adult and 5 fetal human tissues examined, and expression of each gene appears to be independently regulated. Comparison with fragmentary earlier studies of the expression of several of the same enzymes in rat tissues suggests that the overall pattern of expression is largely conserved.

PMID: 8027041 [PubMed - indexed for MEDLINE]

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Molecular cloning and expression of Gal beta 1,3GalNAc alpha 2,3-sialyltransferase from mouse brain.

Lee YC, Kurosawa N, Hamamoto T, Nakaoka T, Tsuji S.

Frontier Research Program, Institute of Physical and Chemical Research (RIKEN), Wako, Japan.

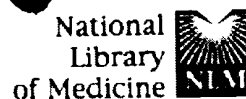
DNA clones encoding beta-galactoside alpha 2,3-sialyltransferase have been isolated from mouse brain cDNA libraries using sequence information obtained from the conserved amino acid sequence of the previously cloned enzymes. The cDNA sequence revealed an open reading frame coding for 337 amino acids, and the deduced amino acid sequence showed 80% identity with that of porcine submaxillary gland Gal beta 1,3GalNAc alpha 2,3-sialyltransferase. The primary structure of this enzyme suggested a putative domain structure, like that in other glycosyltransferases, consisting of a short NH₂-terminal cytoplasmic domain, a signal-membrane anchor domain, a proteolytically sensitive stem region, and a large COOH-terminal active domain. The identity of this enzyme was confirmed by construction of a recombinant sialyltransferase in which the NH₂-terminal part including the cytoplasmic tail, signal-anchor domain and stem region was replaced with an immuno-globulin signal sequence. The expression of this recombinant in COS-7 cells resulted in secretion of a catalytically active and soluble form of the enzyme into the medium. This enzyme exhibited the transferase activity toward only the disaccharide moiety of Gal beta 1,3GalNAc of glycoproteins and glycolipids, no significant activity being detected for the other substrates tested.

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Cloning and expression of the Gal beta 1, 3GalNAc alpha 2,3-sialyltransferase.

Gillespie W, Kelm S, Paulson JC.

UCLA School of Medicine, Department of Biological Chemistry 90024.

Sequence information obtained by NH₂-terminal sequence analysis of two molecular weight forms (45 and 48 kDa) of the porcine Gal beta 1,3GalNAc alpha 2,3-sialyltransferase was used to clone a full-length cDNA of the enzyme. The cDNA sequence revealed an open reading frame coding for 343 amino acids and a putative domain structure consisting of a short NH₂-terminal cytoplasmic domain, a signal-anchor sequence, and a large COOH-terminal catalytic domain. This domain structure was confirmed by construction of a recombinant sialyltransferase in which the cytoplasmic domain and signal-anchor sequence of the enzyme was replaced with the cDNA of insulin signal sequence. Expression of the resulting construct in COS-1 cells produced an active sialyltransferase which was secreted into the medium in soluble form. Comparison of the cDNA sequence of the sialyltransferase with GenBank produced no significant homologies except with the previously described Gal beta 1,4GlcNAc alpha 2,6-sialyltransferase. Although the cDNA sequences of these two enzymes were largely nonhomologous, there was a 45-amino acid sequence which exhibited 65% identity. This observation suggests that the two sialyltransferases were derived, in part, from a common gene.

PMID: 1383214 [PubMed - indexed for MEDLINE]

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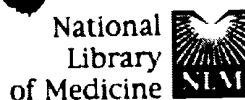
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Molecular cloning and expression of chick Gal beta 1,3GalNAc alpha 2,3-sialyltransferase.

Kurosawa N, Hamamoto T, Inoue M, Tsuji S.

Frontier Research Program, Institute of Physical and Chemical Research (RIKEN), Saitama, Japan.

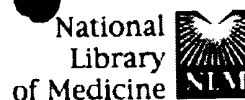
A cDNA clone encoding chick Gal beta 1,3GalNAc alpha 2,3-sialyltransferase (ST3Gal I) was isolated from a chick embryo brain cDNA library. The cDNA sequence included an open reading frame coding for 342 amino acids, and the deduced amino acid sequence showed 64% identity with that of the mouse enzyme. Northern blot analysis of chick embryos revealed that the ST3Gal I gene was expressed in early embryonic stages. The identity of the enzyme was confirmed by construction of a recombinant sialyltransferase in which the N-terminal part including the cytoplasmic tail and signal anchor domain was replaced with an immunoglobulin signal peptide sequence. This enzyme expressed in COS-7 cells exhibited transferase activity similar to that of mouse ST3Gal I.

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Cloning of cDNA encoding the membrane-bound form of bovine beta 1,4-galactosyltransferase.

D'Agostaro G, Bendiak B, Tropak M.

Department of Medical Genetics, University of Toronto.

UDPgalactose: N-acetyl-D-glucosamine 4-beta-D-galactosyltransferase (EC 2.4.1.38) (GalT) is a Golgi-membrane-bound enzyme that participates in the biosynthesis of the oligosaccharide structures of glycoproteins and glycolipids. Synthetic DNA oligomers representing segments of the published partial cDNA sequence for bovine GalT were used as molecular probes to isolate from bovine-liver cDNA libraries overlapping cDNA clones that span 1728 nucleotides and potentially code for the entire polypeptide chain of bovine galactosyltransferase. The cDNA sequence for bovine GalT reveals a 1206-base-pair open reading frame that codes for 402 amino acids, including a presumptive N-terminal membrane anchoring domain of 20 hydrophobic amino acids. The colinearity between the cDNA sequence and 29 non-overlapping amino acid residues which were positively identified by N-terminal sequencing of two polypeptides isolated from the soluble form of the enzyme was consistent with the translation frame and confirmed the authenticity of the cDNA clones. The finding of an N-terminal hydrophobic segment which serves as the membrane anchor and signal sequence suggests that the C-terminal region of the GalT polypeptide is oriented within the lumen of the Golgi membranes. This conclusion is in agreement with previous biochemical studies which indicated that the 51-kDa and 42-kDa soluble forms of the enzyme which encompass the C-terminal 324 and 297 amino acid residues of the entire GalT polypeptide, respectively, include the catalytic site.

PMID: 2502398 [PubMed - indexed for MEDLINE]

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Alpha2,6-sialylation of cell-surface N-glycans inhibits glioma formation in vivo.

Yamamoto H, Oviedo A, Sweeley C, Saito T, Moskal JR.

Chicago Institute for Neurosurgery and Neuroresearch, Chicago, Illinois 60614, USA.

Human gliomas express very high levels of cell-surface alpha2,3-linked terminal sialic acids on glycoproteins bearing N-linked oligosaccharides, most notably on alpha3beta1 integrin, which is the predominant integrin found in these tumors. Alpha2,6-linked terminal sialic acids, however, are not expressed. Two stable transfectants were made using a tumorigenic human glioma cell line, U-373 MG. Galbeta1.4GlcNAc alpha2,6-sialyltransferase (ST6Gal I) transfectants were made to replace the endogenous alpha2,3-linked sialic acids with alpha2,6-linked sialic acids. And Galbeta1.3(4)GlcNAc alpha2,3-sialyltransferase (ST3Gal III) transfectants were made to increase further the expression of cell-surface, N-glycan, alpha2,3-linked sialic acids. Although ST3Gal III transfection resulted in increased invasivity when compared with parental U-373 MG and vector-transfected control cells in vitro, ST6Gal I transfection abolished invasion in vitro and induced alterations in both cell morphology, cell-spreading, and adhesion-mediated protein tyrosine phosphorylation. Furthermore, the ST6Gal I transfectants produced no intracranial tumors in severe combined immunodeficient mice, whereas parental U-373 MG cells, the vector-transfected control cells, and ST3Gal III-transfected U-373 MG cells did. These results suggest that both the linkage and expression levels of the terminal sialic acids of alpha3beta1 integrin N-glycans play an important role in glioma cell-extracellular matrix interactions. Thus, manipulating ST6Gal I gene expression may have therapeutic potential for the treatment of malignant gliomas.

PMID: 11559557 [PubMed - indexed for MEDLINE]

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☐ 2: Cancer Lett 2000 Aug 11;156(2):191-8

RESEARCH ARTICLE
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The identification of novel therapeutic targets for the treatment of malignant brain tumors.

Kroes RA, Jastrow A, McLone MG, Yamamoto H, Colley P, Kersey DS, Yong VW, Mkrdichian E, Cerullo L, Leestma J, Moskal JR.

The Chicago Institute of Neurosurgery and Neuroresearch, 2515 N. Clark St., Suite 800, Chicago, IL, 60614, USA.

A two-step strategy was developed consisting of differential display reverse transcriptase polymerase chain reaction (DDRT-PCR) with cultured normal human fetal astrocytes and U-373MG glioma cells followed by reverse Northern analysis of normal brain and primary tumor tissues. hu-dek, alpha-NAC, ribosomal proteins L7a and L35a, and five novel genes were identified. Since none of these genes has been previously shown to be associated with malignant brain tumor formation, this approach may be useful to identify novel targets for the diagnosis and treatment of brain tumors.

PMID: 10880769 [PubMed - indexed for MEDLINE]

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☐ 3: J Neurochem 1997 Jun;68(6):2566-76

alpha2,6-Sialyltransferase gene transfection into a human glioma cell line (U373 MG) results in decreased invasivity.

Yamamoto H, Kaneko Y, Rebbaa A, Bremer EG, Moskal JR.

Chicago Institute for Neurosurgery and Neuroresearch, Illinois, U.S.A.

Glycosyltransferase gene transfection into cell lines has been an approach used successfully to elucidate the functional role of cell surface glycoconjugates. We have transfected the rat CMP-NeuAc:Galbeta1.4GlcNAc alpha2,6-sialyltransferase (EC 2.4.99.1) gene into a human, tumorigenic, glioma cell line, U373 MG. This transfection led to a marked inhibition of invasivity, alterations in adhesivity to fibronectin and collagen matrices, and inappropriately sialylated alpha3beta1 integrin. Adhesion-mediated protein tyrosine phosphorylation was reduced in the transfectants despite increased expression of focal adhesion kinase, p125fak. Furthermore, the transfectants showed a distinct cell morphology, an increased number of focal adhesion sites, and different sensitivity to cytochalasin D treatment than control U373 MG cells. These results suggest that inappropriate sialylation of cell surface glycoconjugates, such as integrins, can change focal adhesion as well as adhesion-mediated signal transduction and block glioma cell invasivity in vitro.

PMID: 9166754 [PubMed - indexed for MEDLINE]

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4: Brain Res 1997 Apr 25;755(1):175-9

FULL-TEXT ARTICLE

alpha2,3-sialyltransferase mRNA and alpha2,3-linked glycoprotein sialylation are increased in malignant gliomas.

Yamamoto H, Saito T, Kaneko Y, Kersey D, Yong VW, Bremer EG, Mkrdichian E, Cerullo L, Leestma J, Moskal JR.

The Chicago Institute of Neurosurgery and Neuroresearch, IL 60614, USA.

CMP-NeuAc:Galbeta1.3(4)GlcNAc alpha2,3-sialyltransferase (alpha2,3-ST) mRNA was expressed in human glioma specimens, human fetal astrocytes, and a panel of brain tumor cell lines. Maackia amurensis agglutinin staining revealed the presence of alpha2,3-linked sialic acids on glioma cell surfaces and extracellular matrices whereas normal human adult astrocytes were negative. Increased expression of alpha2,3-linked glycoprotein sialylation may play a role in glial tumorigenesis.

Publication Types:

- Clinical Trial
- Controlled Clinical Trial

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5: Acta Neuropathol (Berl) 1996;91(3):284-92

 **pathologica**

The expression of Gal beta 1.4GlcNAc alpha 2,6 sialyltransferase and alpha 2,6-linked sialoglycoconjugates in human brain tumors.

Kaneko Y, Yamamoto H, Kersey DS, Colley KJ, Leestma JE, Moskal JR.

Chicago Institute for Neurosurgery and Neuroresearch, IL 60614, USA.

CMP-NeuAc:Gal beta 1.4GlcNAc alpha 2,6 sialyltransferase (alpha 2,6-ST) [EC 2.4.99.1] is developmentally regulated, shows a high degree of tissue specificity, and appears to play a role in oncogenic transformation and metastasis. In the present study, we have performed the first detailed analysis of the expression of alpha 2,6-ST and alpha 2,6-linked sialoglycoconjugates in human brain tumors. We used a polyclonal, monospecific anti-rat alpha 2,6-ST antibody and the alpha 2,6-linked sialic acid-specific lectin, Sambucus nigra agglutinin (SNA) for histochemical studies, and a human alpha 2,6-ST-specific cDNA probe for Northern analysis. Meningiomas, chordomas and craniopharyngiomas frequently expressed alpha 2,6-ST and alpha 2,6-linked

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different meningioma subtypes. Meningothelial meningiomas stained more
T antibody and SNA than the fibroblastic and anaplastic meningiomas. On
igin and medulloblastomas were virtually devoid of either alpha 2,6-ST or
expression. Moreover, very weak to negligible expression of both alpha
oconjugates was observed in brain metastases. In conclusion, alpha 2,6-ST
te expression is associated with non-neuroectodermal epithelial-like

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**1,4GlcNAc alpha 2,6 sialyltransferase [EC 2.4.99.1]
sialic acids in human brain tumours.**

D. Mkrdichian E. Cerullo L. Leestma J. Moskal JR.

IL 60614, USA.

1,4GlcNAc alpha 2,6 sialyltransferase (alpha 2,6-ST) [EC 2.4.99.1]
2,6-linked sialic acids were examined in primary human brain tumours and cell
meningiomas expressed alpha 2,6-ST mRNA. 42% (10/24) of which showed very
alpha 2,6-ST mRNA expression was undetectable in normal brain tissue. In contrast, only 1/13
was examined expressed detectable alpha 2,6-ST mRNA. Metastases to the brain did not express
asurable amounts of alpha 2,6-ST mRNA. Less expression was found in malignant (i.e. anaplastic) compared
to benign (i.e. meningothelial) meningiomas. Two-dimensional SDS-PAGE of glioma and meningioma
proteins, followed by Sambucus nigra lectin staining, revealed the presence of a glycoprotein bearing alpha 2,6-
linked sialic acids, M(r) = 53 kDa and a pI = 7.0 (MEN-1) that appeared in all seven of the meningiomas
examined, but was expressed at barely detectable levels, if at all, in seven out of the seven glioblastomas
examined. Thus, decreased alpha 2,6-ST expression may play a role in the aggressive nature of anaplastic
meningiomas, but appears to be virtually absent in all tumours of glial origin.

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